Insect pest control and health

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INSECT PEST CONTROL AND HEALTH

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Preface

This paper presents current and future research on insects carried out in the Group Sensory Organs and Behaviour. It describes studies on tsetse flies, mosquitoes, and house- and blowflies, insects that have a severe impact on human and animal health and economic development in many developing countries, particularly in Africa. Tsetse flies and mosquitoes are the main vectors of sleeping sickness and malaria; the ubiquitous house- and blowflies are not only a nuisance by their presence, but also transmit a wide variety of diseases which threaten the lifes of thousands of people.

The research of the group involves studies on the responses of insects to odours and visual stimuli, which enable the insects to localise their hosts from a distance, and on the responses to taste, thermal, and tactile stimuli, which may operate after contact has been achieved. The behaviour of the insects and the electrical activities occurring in their sense cells during stimulation are studied. The sensory activities provide direct information about the specificities and sensitivities of the sense cells and allow a rapid separation of biologically active and non-active stimuli. In this way stimuli that play a role in intra- and interspecific communication can be identified. These stimuli may be used to manipulate the insects' behaviour in such a way as to achieve effective, environmentally-friendly and sustainable insect control. The research aims to develop attractive traps and insecticide-impregnated screens and insect repellents for sustainable use by the local people in rural communities.

The research is carried out in co-operation with African and European institutes and will contribute to improvement of the health status of people and to sustainable improvement of animal husbandry in developing countries.

General Introduction

This paper deals with joint research carried out by the Group Sense Organs and Behaviour (GSOB) of the Department of Animal Physiology of the University of Groningen (RUG) together with institutes in Sub-Saharan Africa and institutes in The Netherlands and other countries in Europe.

The research aims at improving human, animal and plant health and well-being by controlling insect pests and insect vectors of diseases. The studies focus on the role of insect sense organs in causation and maintenance of behaviour in insects. Most of the studies concentrate on the sensory and behavioural responses to odours and visual stimuli which enable the insects to localise their hosts from a distance. However, we also investigate the responses to taste, thermal, and tactile stimuli, which may operate after contact has been achieved. We examine if and how sensitivity differs between insect species and sexes and varies with their physiological state (age, starvation, pregnancy). Diel activity rhythms of insects, in the laboratory as well as in the field, are recorded to determine when the sensitivity to stimuli may be optimal. Mobile equipment is used to record the activities of insect olfactory cells in the field to determine the spatial and temporal distribution of odours in the environment.

This knowledge provides more insight into the fundamentals of sensory processing and also helps to identify stimuli that play a role in intra- and interspecific communication and may be used to manipulate the insects' behaviour in such a way as to achieve effective, environmentally-friendly and sustainable insect control.

GSOB-RUG has a long history of work on insects: moths, caterpillars and flies, several of them being pests in developing countries. At the moment research involves laboratory and field studies on chestnut moths (*Cydia* spp.), which are the most important insect pests of chestnut fruits in Southern Europe and Asia; fruitflies (*Drosophila* spp.), which are pest insects in viniculture; tsetse flies (*Glossina* spp.), the vectors of African human and animal trypanosomosis; mosquitoes (*Anopheles* spp.) which are vectors of malaria; and the ubiquitous house- and blowflies (*Musca*, *Fannia*, *Calliphora*) which are a nuisance to man and animals and are potential vectors of many diseases all over the world.

GSOB-RUG has long experience in research and training activities in an African environment. From 1981 to 1984 the research potential of the Sensory Physiology Group of the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi (Kenya) was increased by building new setup and training of staff. In 1985 and 1986, lectures in animal physiology were given at the University of Ouagadougou (Burkina Faso), and from 1992 to 1995 at the

Universidade Eduardo Mondlane, Maputo (Mozambique). In addition, from 1991 to 1995, GSOB-RUG was involved in backstopping of ecological and parasitological activities of the first phase of a cooperative project between the University of Groningen and the University of Maputo. Now it participates in the Supporting Group Tropical Ecology which gives scientific and educational advice to the second phase of this project, which will be continued till the year 2000.

From October 1985 to July 1987 the group was involved in a joint research project on control of tsetse flies of the European Commission DG XII, Subprogramme Tropical and Subtropical Agriculture, together with the Institut d'Elevage et de Médecine Vétérinaire des Pays Tropicaux (IEMVT) in Maisons-Alfort, France. This collaboration has led to a PhD thesis of a student of IEMVT and five joint papers about the taste and olfactory organs of tsetse flies (morphology, distribution, ultrastructure, function).

From April 1993 to July 1996 the group took part in the Regional Tsetse and Trypanosomiasis Control Programme of Malawi, Mozambique, Zambia and Zimbabwe (RTTCP), Phase II. Experiments were done on tsetse flies both in the field in Zimbabwe and in our laboratory in The Netherlands. GSOB-RUG still cooperates with RTTCP in Harare, and with the Chemical Ecology Group of Natural Resources International Ltd (NRI) in Chatham, England. NRI was also involved in RTTCP, Phase II. It is an internationally recognized centre of expertise in the identification of naturally occurring chemicals that are important for inducing host-seeking behaviour; it has great experience in developing countries;.

Close links still exist with the University of Ouagadougou. This university has worked closely together with the RUG since 1985 in an inter-university training programme, financed by the Netherlands Ministry of Foreign Affairs, Development Co-operation. Various areas of education and research have been and are covered: didactic support in mathematic and physical sciences (1979-1985), education and research in ecology (since 1983) and animal nutrition (since 1985). In GSOB-RUG two students from the Faculté des Sciences et Techniques of the University of Ouagadougou (FAST-UO) have been trained in sensory physiology. This has resulted in two joint papers.

Contacts also exist with The Centre International de Recherche-Développement sur l'Elevage en zone subhumide (CIRDES) in Bobo-Dioulasso, Burkina Faso. CIRDES is a regional Centre with an international charter. Its scientific programme is focussed on the control of tsetse flies to improve health and production. It is conveniently situated at a crossroad between several countries within the subhumid zone where extensive agricultural development is expected.

In Europe joint research is undertaken on tsetse flies with the Département d'Elevage et de Médecine Vétérinaire (EMVT) of the Centre de Co-opération International en Recherche Agronomique pour le Développement (CIRAD), in Montpellier, France. CIRAD-EMVT concentrates on the improvement of health of livestock and on livestock production by protection against major tropical diseases.

Collaboration also exists with the Dipartimento di Scienze Animali, Vegetali e dell'Ambiente of the Università degli Studi del Molise, Campobasso (Italy). This has led to a joint paper and a PhD thesis of a scientist of the Italian university dealing with the behaviour of chestnut moths and electrophysiological properties of their olfactory cells. Recently, cooperative studies have started on the electrophysiology of single olfactory cells of fruitflies. Exchange of scientists and students between the RUG and the University of Campobasso takes place.

In The Netherlands, GSOB-RUG is coordinator of a joint research programme on mosquitoes carried out together with the Agricultural University of Wageningen. In addition, it coordinates a joint research programme on house- and blowflies executed together with the Department of Biophysics and the Department of Pharmacochemistry and Molecular Pharmacology of the RUG. In the latter programme 4 industries take part: Denka International B.V., Barneveld; Berson Milieutechniek B.V., Nuenen; Afdeling Speciale Fluorescentielampen, Philips, Roosendaal; and Syntech, Hilversum.

Collaboration between the GSOB-RUG and institutes in Africa is attuned to enhancing the health status of the rural communities by protecting them and their livestock against diseases by development of non-pollutant, sustainable, low-cost techniques for controlling the insect vectors. Research capacity in the developing countries will be increased by training personnel in the region in techniques to record the activities from sensory organs and the daily rhythms of insects to be used both in the laboratory and the field. Mutual exchange of knowledge will occur and nuclei of European as well as African scientists will be constituted who have acquired the necessary knowledge to continue future research in these important fields. In addition, dissemination of knowledge to the local population will be undertaken.

In the following attention will be paid to studies on tsetse flies, malaria mosquitoes and houseflies, insects that have severe impact on human and animal health in Africa and other developing countries.

1 Research on Tsetse Flies (*Glossina* spp.)

1.1 Introduction

African trypanosomosis is induced by trypanosomes, unicellular organisms, which are transmitted by tsetse flies (*Glossina* spp). Human trypanosomosis ("sleeping sickness") is a predominant disease in Sub-Saharan Africa. Each year, an estimated 250,000-300,000 new cases of human trypanosomosis are reported, the majority in West and Central Africa. However, since trypanosomosis is a disease of rural Africa many sufferers go undiagnosed and thus untreated. The World Health Organisation estimates that every year another 250,000-300,000 women, men and children are left to suffer for lack of diagnosis and treatment. For untreated cases, death is not a risk but a certainty.

Animal trypanosomosis ("nagana") is the major vector-borne disease and most important limiting factor in development of the livestock sector in Africa. With more than 50 million cattle at risk of becoming infected, the costs due to direct losses in animal production and reproduction caused by morbidity, mortality and infertility and for tsetse fly/trypanosomosis control interventions are estimated to be more than US$ 500 million annually.

So far, immunization against trypanosomosis is impossible, because of antigenic variation of the trypanosomes, which outwits the animal and human immunological defence systems. Therapeutical use of drugs is costly; repeated treatments and diagnostic facilities are required for the drugs to be used properly. Furthermore, the number of drugs available is limited. Therefore, the control of the disease still largely depends on fighting the vector: the tsetse flies. Destruction of game animals, which are potential reservoirs of trypanosomes, and of tsetse fly habitats (bush clearing) was undertaken, but has now largely ceased, because of ethical and ecological reasons. Nevertheless, low-scale bush clearing is still practised and constitutes a great danger to the environment (desertification). Spraying of insecticides is pollutant and is a high-cost, high-technology tactic. The sterile male technique is only effective against low population densities and mass production of sterile males is cumbersome and expensive. Therefore, control of tsetse flies now largely depends on the attraction of the flies to traps and insecticide-impregnated screens. This is an environmentally-acceptable, relatively cheap, sustainable alternative approach to the above methods.

Although all (more than 30) species of tsetse flies are polyphagous, each species shows host preferences which may be modified depending on host availability. Tsetse flies find their hosts by visual and chemical cues. Shape, orientation, colour, brightness, contrast, and movement are all important properties in the attraction of tsetse flies to traps and screens. However, trapping using visual stimuli only does not suffice for effective control except with a costly, very high density of targets. However, it is now generally accepted that tsetse flies discriminate between hosts on the basis of a specific blend of odour emitted by the hosts. Addition of olfactory attractants from host odours greatly improves the effectiveness of traps and screens for savannah species in East and Southern Africa. Odour-baited devices are now widely used for control of these species. However, for most tsetse species the odorous compounds luring them to their hosts are unknown.

1.2 Proposed joint research

Co-operating partners

GSOB-RUG has prepared a proposal for research on tsetse flies in which will participate: GSOB-RUG, CIRDES, FAST-UO, CIRAD-EMVT, NRI, and the Laboratoire Central Vétérinaire (LCV) in Bamako, Mali. The latter laboratory is established with the aim to develop techniques for controlling tsetse flies that are environmentally-acceptable and appropriate for sustainable use in rural communities.

State of knowledge

The identification of olfactory attractants for the savannah species of tsetse flies occurred in trapping experiments in the field. However, field experiments are often very time-consuming because they are affected by many variables, such as weather conditions and population densities. In fact, it took more than 10 years to identify the attractants for these species. Therefore, to speed things up it is often recommendable to first carry out experiments in the laboratory before going to the more complex situation in the field. In the laboratory, experiments can be done under carefully controlled conditions.

We are able to record the electrical activities occurring in the olfactory cells of the flies. On stimulation with odour components which are smelled by the insects these activities change. These *electrophysiological* studies provide direct information about the specificities and sensitivities of the cells. They offer a rapid method for screening (fractions of) host emanations, extracts or washes for their stimulatory effectiveness on the flies. In this way biologically active and non-active substances can be separated relatively easily and the active substances identified.

The four most important and widespread species of the *palpalis* group of tsetse, *G. fuscipes fuscipes, G. palpalis palpalis, G. p. gambiensis* and *G. tachinoides,* occupy relatively localised, riverine habitats and can be caught using traps or screens of appropriate size and colour (Laveissière *et al.*, 1986; Cuisance *et al.*, 1991). However, trapping using visual stimuli only does not suffice for effective control. Control of savannah species in East and Southern Africa using visual stimuli was also not successful, but addition of olfactory attractants from host odours greatly improved the effectiveness of the targets. Odour-baited devices are now widely used for control of these species (Vale *et al.*, 1988a, b). Addition of attractants to traps and screens for the *palpalis* group could greatly increase the effectiveness of these targets. However, the attractants used for savannah flies - acetone, 1-octen-3-ol, some simple phenols - are hardly or not attractive to the riverine *palpalis* species and sometimes even repel them (Mérot *et al.*, 1988; Späth, 1995; Mwangelwa *et al.,* 1995). Interestingly, electrophysiological studies showed that the flies do smell these substances (Den Otter, 1987; Den Otter & Tchicaya, 1989; Den Otter *et al.*, 1988, 1991; Van der Goes van Naters *et al.*, 1996). These studies also revealed that in *G. f. fuscipes* more than 30% of the olfactory cells do not respond to the attractants for savannah tsetse, suggesting that we are still unaware of odorous substances that may be of importance to the flies (Van der Goes van Naters, 1997).

There is now increasing evidence that *palpalis* tsetse can adapt to changing conditions in their habitats and availability of hosts resulting from a steadily increasing demographic pressure on arable land. In adapting to these manmade changes, these flies became main vectors of animal and human trypanosomosis in various parts of Africa (Van Vegten, 1971a, b; Gouteux *et al.*, 1994; Okoth, 1986). Recently a substantial increase in the number of autochthonous cases of sleeping sickness due to the presence of *G. p. gambiensis* was found along the Black Volta at about 50 km from Bobo-Dioulasso, an important urban centre in Burkina Faso (Bauer, pers. comm.).

Although these species appear to be opportunistic in their host preferences, blood taken from reptiles may constitute the majority of their meals (Van Vegten, 1971a, b; Gouteux *et al*., 1994).

Given (a) the small size of most reptiles, their low population densities and discreet mode of life, and (b) the high proportion of meals taken from them by the riverine tsetse, it has been proposed that emanations from reptiles may attract *palpalis* flies (Cuisance, pers. comm.). Indeed, preliminary studies by CIRDES showed that *G. p. gambiensis* and *G. tachinoides* are readily able to find hidden reptiles. In addition, trapping experiments in the Central African Republic showed that catches of *G. f. fuscipes* significantly increase when the traps contain the monitor lizard, *Varanus niloticus* (Gouteux *et al.*, 1995). Recent observations around Banjul, The Gambia, confirm the attractiveness of monitor lizards for *G. p. gambiensis*, which were seen to feed in large numbers on these reptiles in spite of dense vegetation, rendering them almost invisible (Bauer, pers. comm.). Moreover, studies in GSOB-RUG showed that emanations from *Varanus niloticus* contain components that are smelled by *G. f. fuscipes* (Van der Goes van Naters, pers. comm.).

Despite the strong indications that tsetse of the *palpalis* group use odours to locate hosts, nothing is known of the chemicals involved. This project will establish the role played by odours in host finding by these flies and use modern state-of-the-art techniques for analysis of attractive volatiles, techniques which we have developed during the last few years.

Objectives

This joint research will contribute to improvement of the health status of people and to sustainable improvement of animal husbandry in developing countries. The project concentrates on research on environmentally-safe tools for control of tsetse flies in Sub-Saharan Africa, especially in Western and Central Africa. The research will study the role of (so far unknown) olfactory attractants in host-finding behaviour of riverine tsetse flies of the *palpalis* group with the objective to improve the attractiveness and operational use of traps and insecticide-impregnated screens for these flies. These tools are environmentally-acceptable and appropriate for sustainable use by the local people in rural communities. Insecticides are not sprayed around, but applied in small amounts on the artificial targets (traps and/or screens) only. Moreover, traps can be used without any insecticide (Cuisance *et al.,* 1991). Any effect on non-target insects is hardly found; bees are not affected (Anonymous, 1988).

The outputs of the research will help to increase the cost-effectiveness of approaches using traps and screens for the control of tsetse and trypanosomosis. This method is successfully used for savannah species of tsetse in Kenya and Zimbabwe, where catches of *G. pallidipes* may increase up to 15-20 times and those of *G. morsitans morsitans* 3-4.5 times when traps are baited with odours from their hosts (Owaga *et al.*, 1988, Vale *et al.*, 1988a, b). There, the cost-effectiveness of baits for tsetse control has been increased several hundred times, to the point where bait techniques offer an economical and effective alternative to control measures that rely on the application of insecticides (Vale, pers. comm.). It is anticipated that the cost-effectiveness of baits for the *palpalis* group of tsetse can be improved to a similar extent when suitable odour attractants become available. Local people will be made aware of the concept of sustainable insect control and environmental protection and will be trained and involved in the implementation of these techniques. In addition, institution-building in the region will be undertaken.

A self-sustaining, community-based environmentally-safe control technique will be established that protects the rural community and their livestock against trypanosomosis, thereby enhancing the health status of the people, the capacity for food production and the productivity of the current livestock.

Specific attention will be paid to

* Establishing the role of olfaction in host-finding behaviour of tsetse flies of the *palpalis* group and determining the most attractive host species emanating tsetse attractants.
* Identifying components of host odours responsible for attraction.
* Developing lures based on blends of these attractants for field use and evaluating their performance with traps and screens in the field.
* Investigating when and how tsetse flies are stimulated by odours in the field in order to determine optimum placement patterns for odour-baited traps and screens.
* Making facilities and expertise available for training collaborating scientists in the region.
* Promoting implementation of the outcome of the research.

Detailed description of the research work

Collection of host odours - Animals that emanate attractants for *palpalis* tsetse (*Glossina p. gambiensis, G. tachinoides*) in the region will be identified. Primarily, we will concentrate on reptiles since the proportion of blood meals taken from reptiles may constitute the majority of the meals of *palpalis* tsetse. One species of animal will be hidden in a pit and the total odour blown towards a screen. Attracted flies will be electrocuted by electrified grids. In addition, using an airtight bag, the attractiveness of certain parts of the body of attractive animals - head (breath), thoracic, abdominal or anogenital region - will be determined by selective exposure. Catches will be compared with those of unbaited screens and with visible control animals. Since the existence of subpopulations with different feeding preferences cannot be ruled out, the responses of flies to odours of man and to the odours found to attract species of the *morsitans* group, will also be determined.

Field entomologists and chemists will cooperate to collect host odours in a way that will allow the odours to be stored and transported to the laboratory. Total odours of attractive animals and/or their excrements will be drawn through a filter and trapped on polymers. Extracts will be made of various glands of the animals which proved to be attractive. The skin of these animals will be washed with hydrophilic and/or lipophilic solvents. The filters, extracts and washes will be taken/sent to Europe for electrophysiological and chemical studies.

Identification of host odours - The odours of hosts present in the polymer filters, the extracts and washes will be desorbed thermically and/or washed out, and the components will be separated by a gas chromatograph (GC) . The odour stream from the outlet of the GC will be split and one part led to the detector of the GC and the other part to the antennae of a fly of the *palpalis* group. The GC signals and the activities of the antennal olfactory cells are displayed on-line by a personal computer and compared. In this way, substances that can be smelled by the flies (eliciting responses in the olfactory cells) can immediately be recognized. Our techniques allow long-lasting experiments with a living fly so that a large number of stimuli can be tested.

The identity of the active substances will be established by gas chromatography/mass spectrometry (GC/MS)and samples of these chemicals will be synthesized for studies on their effects on the behaviour of the flies and for trapping experiments in the field.

We will investigate the responses of the olfactory cells of the flies to different concentrations of individual mammalian odours and to components from reptile odours. Furthermore, the responses to odour blends will be studied to investigate eventual mutual inhibitory or synergistic effects of the various odour components on the activities of the cells. Since, ideally, all individuals of a population should respond to the stimuli for odour-baited targets to be optimally effective, the effects of sex, age, starvation, pregnancy and circadian rhythms on olfactory responsiveness will also be determined. Diel patterns of activity of the flies will be recorded with a new type of activity metre fitted with a microwave-Doppler-motion detector. This equipment is already successfully used in the laboratory for determining the circadian rhythms of flies and moths.

Trapping and physiological experiments in the field - Field entomologists will test the effectiveness of various types of traps and screens. They will cooperate with chemists in designing and testing the efficacy of various methods of dispensing odours that were shown to be of practical use. For the composite behaviour of host-finding to occur a multi-component mixture of odours is very likely to be required. Moreover, different doses of odours may have different effects on behaviour (Den Otter, 1991b). Therefore, the candidate attractants will be tested at a range of doses, alone or in mixtures with other components.

Diel patterns of activity of the flies will be recorded under natural conditions in the field, using the new type of activity metre, which can also operate on batteries. Laboratory and field results will be compared. The effects of weather conditions on locomotor activities will be studied and the relationship between, on the one hand, these activities and, on the other hand, the numbers of flies caught and the responses of their olfactory cells determined.

Recently developed portable electrophysiological equipment allows studies of the responses of olfactory cells of the flies in the field at various distances from a host or an artificial odour source (Voskamp *et al.*, in press). This shows when and how tsetse are stimulated by odours in their natural habitat and may shed more light on the way in which tsetse navigate in odour plumes. It may also suggest methods by which odour plumes could be produced more economically and effectively, and help to determine the optimum placement patterns for odour-baited screens and traps. In addition, it could help in establishing the existence of unidentified attractants. As already said before, in *G. f. fuscipes* more than 30% of its olfactory cells do not respond to any of the attractants identified for the savannah species, suggesting that we are still unaware of cues which may be of importance to the flies.

Most field experiments will be carried out in the dry season. Part of the experiments will be done during the raining season to take into account variations in age structures of given tsetse populations resulting from changed climatic conditions (higher emergence rates of flies, etc.). Results obtained in the dry-cool, the dry-hot and the raining season will be compared to get insight into the effects of environmental factors (wind, temperature, humidity: *cf*. Brady *et al.*, 1989; Williams *et al.*, 1990) or physiological factors of the flies (*cf*. Brady, 1972; Langley & Wall, 1990; Den Otter *et al.*, 1991).

Training - Counterpart scientists and students in the region will be trained in ecological and trapping experiments, gas chromatography, and in electrophysiological and activity recordings both in the laboratory and in the field. A scientist of CIRDES will visit the GSOB-RUG to be trained in electrophysiology. A PhD student of FAST-UO will be in Europe to be trained by GSOB-RUG and NRI in electrophysiology and gas chromatography, and carry out experiments in these fields. In addition, this student will perform field experiments at CIRDES.

At the end of the project, the electrophysiological and GC equipment used by GSOB-RUG will be transferred to FAST-UO to be used by the (trained) scientists and by students. This equipment is also suitable for studies on the olfactory sensitivities of other pest insects, either feeding on animals, plants or humans, and for identifying behaviour-modifying chemicals for management of insect pests. A scientist of GSOB-RUG will visit FAST-UO for a period of about 2 months to assist in installing the equipment at FAST-UO and get this equipment working. After the project GSOB-RUG will maintain contacts with FAST-UO in the context of already existing cooperative projects between these two partners.

Implementation/exploitation - CIRDES will assume a key role in the dissemination of knowledge through seminars, workshops and on the job training. All other partners will assist in this task. As soon as results warrant this, written instructions will be presented to the local population in English and French. Regional authorities will be invited and strategies for rapid dissemination of knowledge will be discussed. A manual of instruction, in English as well as French, will be prepared and distributed at the end of the project. CIRDES and FAST-UO will maintain contacts within the region. Regional people trained during the project will advise local communities to manage the control techniques on their own.

Duration of the project - The programme is envisaged to be completed in a relatively short period of three years, since several of the various research activities are carried out simultaneously. On the other hand, the logical sequence of time-consuming activities (chemical identification and synthesis of the odour components, and testing of the candidate attractants in the field) necessitates a project duration of three years.

The project is cost-effective because the most important species of the *palpalis* group will be studied in one and the same project and synergy can be expected.

1.3 Investigations done so far

Studies on tsetse flies in the GSOB-RUG date back to 1984. They concentrated on the role of the senses of the flies in the flies's search for blood. We sought to discern the function of the olfactory, taste and temperature receptors by coupling their electrical responses to the behaviour of the flies. Experiments were done with *Glossina m. morsitans* and *G. pallidipes* of the *morsitans*-group, and *G. f. fuscipes* of the *palpalis*-group tsetse.

As to the responses to odours we recorded the electrical activities of the olfactory cells of the flies; these cells are present in the flies' antennae. We have developed a technique to record so-called electroantennograms (*EAGs*), which reflect the combined electrical activities of many cells in an antenna (Den Otter &

Saini, 1985; Den Otter *et al.*, 1988; Tchicaya, 1990; Den Otter, 1991a; Den Otter *et al.*, 1991). Therefore, EAGs do not provide information about the various types of cells that are activated. However, they can relatively easily be used to detect substances that are smelled by the insect. In addition, we were able to record from individual antennal cells (*single cell recordings*). These recordings provide information about the specificities and sensitivities of the cells responding (Den Otter & Van der Goes van Naters, 1992; Van der Goes van Naters, 1997).

It was found that a small number of the antennal cells are receptors sensitive to temperature changes while the majority respond to one or more of the compounds attractive to tsetse of the *morsitans*-group: carbon dioxide, acetone and 1-octen-3-ol identified in cattle breath, and phenol, 3-methyl-, 3-ethyl-, 3-*n*-propyl-, 4-methyl-, 3-ethyl- and 4-*n*-propylphenol identified in cattle urine. The results revealed which blends of phenols may be most attractive in the field for *G. m. morsitans* (Den Otter & Van der Goes van Naters, 1993). Though *palpalis*-group tsetse flies respond poorly to odour lures used for *morsitans*-group flies, we were able to record responses to all the known attractants in *G. f. fuscipes*. Examination of the responses of the cells sensitive to 1-octen-3-ol revealed that any deviation from the molecular structure of this substance resulted in lower activities (Van der Goes van Naters *et al.*, 1996).

G. m. morsitans bites especially in early morning and late afternoon; around midday feeding is at a low. Locomotion shows a similar pattern of activity levels. We found that the responses of the olfactory cells of this fly to standardized stimuli of host-derived odours fluctuates parallel with these daily rhythms. In contrast to this, the level of spontaneous activity of *G. f. fuscipes* rises gradually over the day. Again, the sensitivity of the olfactory receptors appeared to parallel this species' locomotor activity (Van der Goes van Naters *et al.*, in press).

As to the sense of taste we discovered that two hairs on the distal end of each leg contain cells which are responsive to four (leucine, valine, and uric and lactic acid) of the fourteen major components of human sweat. The flies only show feeding behaviour when surfaces containing these substances are warmed a few degrees above ambient (Van der Goes van Naters & Rinkes, 1993; Van der Goes van Naters *et al.*, in press). Studies on the responses of the taste cells to the twenty common protein amino acids revealed that they are only responsive to eleven of these acids which all excite but one cell in each taste hair (Van der Goes van Naters & Den Otter, in press). Hairs showing the same structure as taste hairs are also found on the wings of the flies (Deportes *et al.*,1994).

Apart from those on the antennae, cells sensitive to temperature changes are present on the legs. Each tarsomere carries two hairs, each of them housing one thermosensitive cell (Van der Goes van Naters, 1997).

A portable device with which both EAG and single-cell recordings can be made, was designed in close co-operation with Syntech, Hilversum (The Netherlands). The device can be taken to the field and carried in one hand, holding it by a handle fixed to its main body. EAGs, the electrical activities from individual antennal cells, and wind speed changes can be monitored acoustically by headphones and stored on tape by a cassette recorder connected to the device.

Recordings under natural conditions in the field from tsetse olfactory cells were done in Zimbabwe. EAGs and single-cell recordings were made from 1-day-hungry females of *G. pallidipes*, the age of which was unknown (Voskamp & Den Otter, 1995a, b).

The recordings in environmental air upwind and downwind of an odour source showed “

“bursts” of responses of olfactory cells. This suggested that odours passed over the antennae in “puffs”. The results show that tsetse flies sense odour as puffs that further downwind of the source on average contain less odour and are passing less frequently.

We propose that averaging odour information over time may be of minor importance in long-range location of odour sources by tsetse. The detection of a single above-threshold odour puff may lead to optomotor-steered anemotaxis. The results suggest that tsetse may smell hosts from 60 m downwind at least and that the number of flies responding to and being caught by odour-baited targets may be higher in woodland than in open field (Voskamp *et al.*, in press).

2 Research on Mosquitoes

2.1 Introduction

Reproduction of most mosquito species depends on blood of vertebrates. Adult females take blood meals for egg development. Malaria is caused by unicellular parasites (*Plasmodium* spp.) which are injected into the body by a bite of a female mosquito. The *Plasmodium* species, with the exception of *P. malariae* (which may affect higher primates) are exclusively parasites of man. The parasites are always transmitted by female *Anopheles* mosquitoes.

At present, at least 300 million people are affected by malaria globally and there are between 1 and 1.5 million malaria deaths per year. Malaria is generally endemic in the tropics with extensions into the subtropics. In 1990, 80% of the cases were in Africa.

The high adaptivity of both the vectors and the parasites is a big problem for controlling the disease; integration of various methods is needed. Therefore, knowledge of both the biology of the vectors and the dynamics of malaria transmission of is needed to produce an appropriate mix of technologies with which to combat the disease.

The closely related species of the *Anopheles gambiae sensu lato* complex show different host preferences: they either are anthropophilic using humans as hosts, zoophilic exclusively biting cattle, or opportunistic showing a varying anthropophilic/zoophilic biting pattern. The level of anthropophily is a major determinant for the vectorial capacity for malaria in these African species (Macdonald, 1957).

The *An. gambiae s. l*. complex consists of six species. *An. gambiae s.s*. and *An. arabiensis* constitute the mass of the *An. gambiae s.l.* population in Africa south of the Sahara (White, 1974). *An. gambiae s.s.* is of great medical importance, being world's most dangerous vector for malaria. It shows a highly selective, anthropophilic biting pattern even when other hosts outnumber men (Boreham, 1975; Garret-Jones *et al*., 1980; Service, 1993). *An. arabiensis* shows an opportunistic, that is, varying anthropophilic/zoophilic behaviour. In most areas in West Africa where its biting preference is studied, its level of anthropophily is comparable to that of *An. gambiae s.s.*, but where cattle is abundant anthropophily is markedly reduced. In South and East Africa *An. arabiensis* females appear to be directed more to feeding on animals. Actually, on Madagascar *An. arabiensi*s populations have been found, which are exclusively zoophilic (White, 1974; Garrett-Jones *et al*., 1980; Gillies & Coetzee, 1987; Colluzzi, 1992). Another member of the complex, *An. quadriannulatus,* is highly zoophilic and therefore not a regular malaria vector. It has a patchy relict distribution in South and East Africa (Gillies & Coetzee, 1987).

Mosquito females searching for a blood meal are able to select their specific host in the middle of other possible hosts (Gillies & Coetzee, 1987). Olfactory cues play a prominent role in their host-seeking behaviour (Bowen, 1991; Takken, 1991).

2.2 Proposed joint research

Cooperating partners

To date GSOB-RUG is scientific coordinator of a research programme carried out in co-operation with the Laboratory of Entomology, Agricultural University of Wageningen, The Netherlands. The programme is entitled "Odour-guided host finding by haematophagous mosquitoes: Recognition of odour patterns". It consists of three projects: 1) "Identification and neural encoding of host odours", 2) "Olfactory basis of anthropophilic and zoophilic behaviour", 3) "The effect of host-specific odours on behaviour". The research started in 1994.

It is proposed to extend this programme as a joint project in which participate the GSOB-RUG, FAST-UO, the Centre National de Lutte contre le Paludisme in Ouagadougou, the Malaria Research and Training Centre of the Département d'Epidémiologie des Affections Parasitaires in Bamako, Mali, and NRI.

State of knowledge

In behavioural studies it was shown that mosquito species with distinct host preferences respond differently to volatile blends from human or animal origin (Gillies, 1967; McIver, 1968; Mukwaya, 1976; Knols 1997) and to single host-odour components (Kline *et al.*, 1990; Takken, 1991; Takken *et al*., 1997). Expired CO_2 is a general activator/attractant for all mosquito species tested so far, but zoophilic and opportunistic species are more responsive to this substance (Snow, 1970; Costantini *et al*., 1996). Furthermore, experiments on biting site selection showed that the anthropophilic species *An. gambiae s.s.* has a preference for biting on the feet of humans, while the zoophilic European species *An. maculipennis atroparvus* has a breath-oriented biting site selection (De Jong & Knols, 1995, 1996). In wind tunnel experiments, *An. gambiae s.s.* is attracted to a synthetic mixture of fatty acids that were identified in foot scrapings (Knols & De Jong, 1996; Knols *et al..* 1997). The fatty acids are breakdown products of human sebum and are thought to contribute to the distinctive human olfactory signature (Nicolaides *et al.*,1968; Stoddart, 1990).

Zoophilic mosquitoes appear to use odours that are more specific to animals. Several field studies (Takken & Kline, 1989; Kline *et al.,* 1990; Van Essen *et al*., 1994; Van den Hurk *et al.,* 1997) have shown that catches of zoophilic mosquitoes increase when traps are baited with 1-octen-3-ol, a substance isolated from cattle breath (Hall *et al.,* 1984). In a wind tunnel, the zoophilic *An. stephensi* is strongly attracted to a blend of CO_2 and 1-octen-3-ol, while *An. gambiae s.s.* does not show behavioural responses to these stimuli (Takken *et al.,* 1997). Phenols, components of cattle urine, known to attract certain species of tsetse flies, may also be attractive to zoophilic mosquito species (Kline *et al.,* 1990).

Electrophysiological research on the responses of the olfactory organs has been used to identify chemicals that are perceived by mosquitoes. Electroantennogram studies showed that certain fatty acids, 1-octen-3-ol as well as phenols indeed evoke responses in *An. gambiae s.s.* antennae (Cork & Park, 1996; Knols *et al.,* 1997). However, more detailed information about the reception of olfactory stimuli can be obtained by single cell recordings.

Objectives

The research aims at determining whether differences exist between the responses of olfactory receptor cells of different mosquito species. It is intended to restrict the studies to the anthropophilic species *An. gambiae s.s.* and the opportunistic species *An. arabiensis*. These studies may contribute to a better understanding of factors involved in host location and selection behaviour of mosquitoes and may provide the fundamental information needed for the development of synthetic attractants or repellents for these mosquitoes. Attractants may be used for improving the effectiveness of odour traps appropriate for monitoring and control of malaria vectors, protecting the rural community against malaria.

Detailed description of the research work

The research aims at determining which odours are involved in host-finding of the species *An. gambiae s.s.* and *An. arabiensis*. The outlines of these studies equals those as described for tsetse flies.

Emanations and washes from man and cattle will be collected, stored and transported to the laboratory. The components will be separated by a gas chromatograph and biologically active substances determined. The identity of these substances will be established and samples of the chemicals synthesized for electrophysiological studies, for studies on the behaviour of the mosquitoes and for trapping experiments in the field.

Responses of olfactory cells of *An. gambiae* and *An. arabiensis* to individual odour substances and odour blends from man and cattle will be compared. Attention will be paid to the effects of the physiological state (pregnancy, "hunger" state, age, circadian rhythms) of the insects on sensory activity.

Field entomologists will cooperate with chemists in designing and testing various methods of dispensing odours that have be shown to be of practical use.

Counterpart scientists and students in the region will be trained in ecological and trapping experiments, gas chromatography, and in electrophysiolgical and activity recordings. Dissemination of knowledge in the region will be undertaken.

2.3 Investigations done so far

We tested olfactory cells of *An. gambiae s.s*., *An. arabiensis*, *An. quadriannulatus* and *An. m. atroparvus* for their responses to fatty acids, 1-octen-3-ol, and 3- and 4-methyl phenol. Olfactory cells in *sensilla trichodea* of all four species respond to these odours. Short-chain fatty acids and phenols may evoke either increase or decrease of "spontaneous" spike activity, whereas stimulation with 1-octen-3-ol results in excitation only. Fatty acids with longer chain lengths (C5, C6, C10, C12, C14) do not evoke responses in these cells.

It was shown that the *Anopheles* species tested differ in their sensitivity to the above "human" and "animal" odours. In *An gambiae s.s.* more cells are found that are excited by fatty acids and these cells are more sensitive to these substances than cells of *An. m. atroparvus* and *An. quadriannulatus*. On the other hand, 1-octen-3-ol evokes significantly lower responses in *An. gambiae* than in the latter two species. *An. arabiensis* shows intermediate responses to these odours. No differences between the responses of the four species to the phenols are found. The results suggest that difference in relative quantities of the various substances emanated by men and animals may determine host choice in these species.

It appeared that fresh sweat from people attractive for mosquitoes does not evoke activity in olfactory cells of *An. gambiae s.s.*, whereas human sweat kept at 37^{o} C for 2 days elicits reproducible responses.

In accordance with the electrophysiological results, it was found that odours from short-chain fatty acids attract *An. gambiae* in a wind tunnel. Fresh human sweat does not evoke behavioural responses, but sweat in which microorganisms (skin flora) had time to decompose substances to volatile components becomes attractive to the mosquitoes. This may account for the many contradictory statements about the attractiveness of human sweat for mosquitoes.

Our hypothesis is that the peripheral sensory cells of malaria species are selective for or highly sensitive to certain odour compounds. Therefore, we are studying the responses of the olfactory cells ànd the behaviour of mosquitoes with different host preferences on stimulation with odours from human and from animal origin.

3 Research on House- and Blowflies

3.1 Introduction

House- and blowflies are cosmopolitan in distribution. Common houseflies (*Musca domestica*) are ubiquitous insects. They are highly active indoors. In warm environments they remain active and reproduce throughout the year. Lesser houseflies (*Fannia canicularis*) are frequently encountered in poultry houses. Blowflies (*Calliphora vicina*) are highly attracted to dead animals, wounds and faeces-caked hair on pets and farm animals. They may gather on door and window screens and enter homes. As with the Common housefly, adults of the latter two species also remain active and reproduce throughout the year in warmer conditions.

These domestic flies are not only a nuisance by their presence, but are also important from a human and animal health standpoint. They can transmit intestinal worms, or their eggs, and are potential vectors of diseases such as conjunctivitis, poliomyelitis, typhoid fever, tuberculosis, leprosy, cholera, diarrhea and dysentery. They frequent and feed indiscriminately on any liquefiable solid food, which may equally be moist, putrefying material or food stored for consumption. While feeding the insects pick up pathogenic organisms which may collect on their bodies to be transferred on contact with other surfaces or survive passage through the gut to be deposited as fly spots.

3.2 Proposed joint research

Cooperating partners

GSOB-RUG is the scientific coordinator of a research programme entitled "Environmentally-safe control of flies using a combination of visual and chemical stimuli". This programme is a joint project of GSOB-RUG and the Groups Visual System Central and Visual System Peripheral of the Department of Biophysics (DB-RUG), and the Department of Pharmaceutical Biology of the Centre for Pharmacy of the RUG (DPMP-RUG). The studies occur in close co-operation with some Netherlands manufacturing enterprises[1], which intend to bring the trap into production when this is opportune. The research started in 1994.

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[1]Denka International B.V., Barneveld; Berson Milieutechniek B.V., Nuenen; Philips International, Roosendaal; Syntech, Hilversum

It is proposed to extend this project as a joint project between the GSOB-RUG, DB-RUG, DPMP-RUG and FAST-UO as part of the inter-university training programme. GSOB-RUG is able to transfer his knowledge about the electrophysiology and behaviour of flies to FAST-UO, whereas the DB-RUG can assist in studies on the flies' visual system. The latter group focuses its attention on properties of retinal photoreceptors (spatial sensitivity, colour sensitivity, temporal solution) and those of motion-sensitive neurons in higher-order ganglia. The DPMP-RUG has a long tradition with respect to collection, identification and synthesis of volatiles present around and in extracts of animal and vegetable materials.

Objectives

As may be clear from the above, flies occur in every continent, including Africa, and are vectors of a wide variety of diseases which threaten the lifes of thousands of people. The programme may provide the fundamentals of processing olfactory and visual information by flies and contribute to the development of synthetic attractants or repellents to be used in fly control techniques.

The research has the practical aim to develop an environmentally-safe trap that is able to catch flies in relatively faintly lighted accommodations (for example, poultry-breedings, cattle-houses, piggeries, but also shops and rooms). The trap has to be more effective than the *UV*-lamps that are presently in practise and has to make use of a combination of attractive visual and olfactory stimuli. The trap should be able to catch both male and female flies of different age and different physiological states.

Detailed description of the research work

The research aims at determining which odours and visual stimuli are involved in host-finding of flies. The outlines of these studies equals those as described for tsetse flies.

Emanations and washes from man and cattle and their excretions, and from food sources of the flies will be collected, stored and transported to the laboratory. The components will be separated by a gas chromatograph and biologically active substances determined. The identity of these substances will be established and samples of the chemicals synthesized for electrophysiological studies, for studies on the behaviour of the mosquitoes and for trapping experiments in the field.

Responses of olfactory cells of the flies to individual odour substances and odour blends will be compared. Attention will be paid to the effects of the physiological state (pregnancy, “hunger” state, age, circadian rhythms) of the insects on sensory activity.

Field entomologists will cooperate with chemists in designing and testing various methods of dispensing odours that have be shown to be of practical use.

Counterpart scientists and students in the region will be trained in ecological and trapping experiments, gas chromatography, and in electrophysiolgical and activity recordings. Dissemination of knowledge in the region will be undertaken.

3.3 Investigations done so far

We try to identify stimuli that evoke and maintain positive behavioural responses (activation, approaching behaviour, probing) in flies. Major attention is paid to the role of the olfactory and visual systems in the causation of behaviour, *i.e.* to the way in which the flies use visual and olfactory stimuli to find their food, hosts, mates and oviposition sites. The studies concentrate on the species mentioned above: the Common housefly, *Musca domestica*, the Lesser housefly, *Fannia canicularis* and the blowfly *Calliphora vicina*.

The responses of the olfactory cells and the behavioural responses of the flies of different physiological state are studied during stimulation with (mixtures of) odours originating from conspecifics and food sources, and from man and animals and their secretions. In addition, the effects of light stimuli of different wave-lengths, intensities, frequencies, modulation-depths and planes of polarisation on the behaviour of the flies are studied. Finally, the behavioural responses to combinations of the most effective visual and olfactory stimuli are investigated.

So far, extensive information about the results of these studies cannot be given, because secrecy has been enjoined upon us by the industries involved.

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